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Mechanism of the Preferential Block of the Atrial Sodium Current by Ranolazine

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Introduction: Atrial-selective inhibition of cardiac sodium channel has been shown to underlie the effectiveness of ranolazine in the suppression of atrial fibrillation. This study investigates the mechanism responsible for differences in the interaction of ranolazine with sodium channels in atrial vs ventricular myocytes. **Methods:** Whole cell sodium current was recorded at 15^0C in isolated canine atrial and ventricular myocytes, and in *HEK*293 cell line expressing SCN5A. Protocols were designed to determine interaction of ranolazine with resting, inactivated, and open states of sodium channels. Single pulses and trains of 40 pulses were elicited over a range of holding potentials in the absence and presence of ranolazine to yield tonic and use-dependent block. Development of block during pulse trains in myocytes was analyzed using guarded-receptor theory. **Results**: Tonic block was negligible at holding potentials up to -100 mV, suggesting minimal drug interaction with resting and inactivated states. However, use-dependent block was increased with more depolarized holding potentials, indicating ranolazine trapping by the inactivation gate. Train protocols demonstrated significant effect of shorter diastolic intervals to increase use-dependent block, but a lack of effect of longer pulse durations. Effects in atrial and ventricular myocytes, and in *HEK293* cells followed a similar pattern.

Conclusions: Ranolazine is a potent open-state blocker of sodium channels that unbinds from the resting channels unusually fast and is trapped in the inactivated state. Kinetic rates of ranolazine interaction with different states of atrial and ventricular sodium channels are similar. Ranolazine inhibition of sodium-channels is atrial-selective due to a more negative position of the steady-state inactivation curve, more positive resting membrane potential, as well as more positive take-off potential and shorter diastolic interval in atrial vs ventricular myocytes at fast rates.

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Determination Of The Specificity For FHF/Na Channel Interactions

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Specific FHFs (Fibroblast growth factor homologous factors) regulate voltage-gated sodium channel (VGSC) function by modulating activation, inactivation and/or subcellular distribution through binding to the C-terminus (CT) of specific VGSC alpha subunits. The effect upon VGSC function varies greatly depending upon the particular FHF isoform and its VGSC binding partner, but the specificity of these pair wise interactions are not understood. To identify the determinants for interaction between specific FHFs and VGSC pairs, we studied binding in a recombinant bacterial co-expression system. We show that FHF1b (aa 1- 144), which contains the conserved FHF β-trefoil core domain and is 60-70% homologous to the other members of the FHF family, bound to a $Na_V1.5$ CT containing aa 1773-1940, but not to a $Na_V1.5$ CT comprised of aa 1773-1878. Since the part of the CT necessary for binding contains the calmodulin (CaM) interaction site, we tested whether CaM and FHF1b competed for binding and found that all three components could form a complex; thus, CaM and FHF do not compete for interaction with the $Na_V1.5$ CT. A full length FHF1b and FHF2b also bound to the $Na_V1.5$ CT (aa 1773-1940) but not $Na_V1.5$ CT (aa 1773-1878), suggesting that the region between aa 1878-1940 is necessary for FHF interaction. FHF1b also bound to $Na_V1.6$ (aa 1767- 1926), but did not bind $Na_V1.1$ (aa 1787-1948) nor $Na_V1.2$ (aa 1772-1937). Since the distal regions among the tested $Na_V1.x$ CTs are less similar than the proximal regions, we predict that the specificity for interaction and the consequent particular effects upon VGSC function derives at least in part from this less-well conserved $Na_V1.x$ region.

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Role of L1462 in $Na_v1.5$ Channel DIII-S6 in Voltage-dependent Gating and Antiarrhythmic Block

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Mutations of domain III S6 (DIII-S6) Leu-1465 of rat brain IIA Na^+ channel ($Na_v1.2$) or the equivalent (L1280) in the adult rat skeletal muscle isoform ($Na_v1.4$) affect local anesthetic and anticonvulsant block (Yarov-Yarovoy et al., 2001; Nau et al., 2003). We examined the role of the equivalent position (L1462) of the human heart isoform ($Na_v1.5$) in voltage-dependent gating and antiarrhythmic block. Whole-cell Na^+ currents were measured in HEK293 cells transiently expressing the recombinant wild type (WT) or mutant channels and the beta-1 subunit. Compared to WT, all the mutants of L1462 (L1462A, L1462C, L1462F) accelerated the current decay and shifted the voltage dependence of activation towards more positive direction. L1462 mutants had no effects on the voltage dependence of fast inactivation and the recovery from fast inactivation, but increased the fraction of intermediate inactivation component. Internal charged methanethiosulfonate, MTSES blocked L1462C/C373F with a high frequency of stimulation (20 Hz), where C373 is known to face the outer pore and be the critical residue for isoform differences in tetrodotoxin block. L1462F had no obvious effects on tonic block, but increased use-dependent block by the class Ic antiarrhythmic drug, flecainide, which binds preferentially to activated channels. L1462F increased affinity of activated channels for flecainide by 4-fold, while L1462F had little effects on affinity of inactivated channels. For internal membrane-impermeant QX314, L1462F increased use-dependent block and affinity for QX314 binding to activated channels by 5-fold. These results suggest that L1462 faces the pore in the open state and is involved in activated channel block by antiarrhythmic drugs as well as inactivated channel block by local anesthetics.

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Regulation Of Cardiac Na^+ Channel By NAD^+/NADH

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Introduction: Glycerol-3-phosphate dehydrogenase 1-like (GPD1-L) mutations have been shown to reduce cardiac Na^+ current and cause Brugada Syndrome. Our previous work suggests that mutations in GPD1-L act through NAD(H) to alter Na^+ current. NADH results in downregulation of Na^+ current and NAD^+ can reverse the downregulation of Na^+ current by mutant (MT) GPD1-L or NADH. Here, we studied potential signaling pathways between GPD1-L, NAD(H), and Na^+ channel.

Methods: Currents were measured using whole-cell patch clamp of HEK cells stably expressing the human cardiac sodium channel with and without chelerythrine (a PKC inhibitor, IC_{50} = 660 nM), $PKAI_{6-22}$ (a PKA inhibitor, IC_{50} = 1.6 nM), apocynin (a NADPH oxidase inhibitor), or elevated intracellular Ca^{2+} in the pipette solution. MT GPD1-L (A280V) was co-transfected with red fluorescent protein with Fugene6 to HEK cells 40 hours prior to measuring current.

Results: The 2-fold reduction in Na^+ current mediated by NADH (100 μM) was inhibited by 200 μM apocynin (50 ± 6% vs. 86 ± 15% of the control current, n=14, $p<0.001$), 6 mM $[Ca^{2+}]_i$ (107 ± 17% of control, n=12, $p<0.001$), or 10 μM chelerythrine (86 ± 14% of control, n=10, $p<0.05$). $PKAI_{6-22}$ blocked the NAD^+-mediated upregulation of Na^+ currents in the presence of MT GPD1-L (n=10, $p<0.001$). Chelerythrine (10-50 μM) or $PKAI_{6-22}$ (50 nM - 5 μM) alone did not affect the peak currents of Na^+ channels.

Conclusions: Our experiments suggest that NAD(H) can alter Na^+ currents. Downregulation by NADH seems to involve the NADPH oxidase and PKC. PKA appears to be involved in NAD^+-dependent current upregulation. This implies that redox/metabolic state can influence Na^+ current.

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Differential Regulation Of Navß Subunits During Myogenesis

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Voltage-gated sodium channels (Na_v) consist of a pore-forming alpha subunit ($Na_v\alpha$) associated with beta regulatory subunits ($Na_v\beta$). Adult skeletal myocytes primarily express $Na_v1.4$ channels. We found, however, using neonatal L6E9 myocytes, that myofibers acquire a $Na_v1.5$-cardiac-like phenotype efficiently. Differentiated myotubes elicited faster $Na_v1.5$ currents than those recorded from myoblasts. Unlike myoblasts, I_{Na} recorded in myotubes exhibited an accumulation of inactivation after the application of trains of pulses, due to a slower recovery from inactivation. Since $Na_v\beta$ subunits modulate channel gating and pharmacology, the goal of the present work was to study $Na_v\beta$ subunits during myogenesis. All four $Na_v\beta$ ($Na_v\beta1$-4) isoforms were present in L6E9 myocytes. While Navβ1-3 subunits were up-regulated by myogenesis, $Na_v\beta4$ subunits were not. These results show that *$Na_v\beta$* genes are strongly regulated during muscle differentiation and further support a physiological role for voltage-gated Na^+ channels during development and myotube formation. Supported by BFI2002-00764 and BFU2005-00695 (to AF), SAF2004-06856, SAF2007-65868 and FIS RD06/0014/0006 (to CV). MD hold a FIS